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FACILITY FORM 602

N65-20981 (ACCESSION NUMBER)	(THRU)
9 (PAGES)	1 (CODE)
CR-53457 (NASA CR OR TMX OR AD NUMBER)	04 (CATEGORY)

(NASA CR-53457)

SEMI-ANNUAL REPORT, ~~TO~~

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

UNPUBLISHED PRELIMINARY DATA

(NASA ~~Research~~ Grant NsG-528)

Effect of Space Environment on Circadian Rhythms of Plants, For the Purpose of Defining and Verifying an Experiment Suitable for Use in a Biosatellite

September 1, 1963 ~~to~~ March 1, 1964

GPO PRICE $ ______

OTS PRICE(S) $ ______

Hard copy (HC) $1.00

Microfiche (MF) $.50

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March 1, 1964 9p refs

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SEMI-ANNUAL REPORT

Grant NsG 528

Research Performed.

INTRODUCTION

During the first six months of the grant period, several new techniques and ideas have developed which appear to enhance the feasiability of the experiment. The size of the plant has been reduced, a new recording system is being tried out and a possible axenic culture technique is being developed. In addition to these recent developments, the Nogravatron, a weightlessness simulating apparatus developed in our laboratory, has been tested. Quick growing Atlas barley seedlings were placed on the Nogravatron and growth rates were compared to stationary controls. Striking difference were observed in coleoptile and first leaf elongation rates. Typical responses are depicted in Fig. 1, 2, 3. It is hoped to test and record in the very near future Pinto bean leaf movements under simulated weightlessness conditions by growing and photographing plants on the Nogravatron. The purchased time-lapse camera and accessory equipment arrived recently and the shutter triggering mechanism is being adapted to the Nogravatron.

PINTO BEANS

Confirmation of Data.

Additional stationary control data for the primary leaves of Pinto beans have been obtained by the Ames Research Laboratory and has confirmed previous findings on Pinto bean leaf movements.

Size Reduction of Experimental Package.

A decrease in the ultimate size of the experimental container to 3" x 3" x 12" has been brought about as mentioned before by the technique of using detached primary leaves. Time lapse recording has shown that there are no significant changes in the rhythmicity of the detached leaf from that of leaves attached to whole plants. Under greenhouse conditions, detached leaves which were rooted in vermiculite have been transplanted and grown in potting soil for as long as 6 weeks. Additional fluorescent lamps and accessories have been purchased to study in more detail the leaf movement phenomenon. Work is progressing on the cultural technique of maintaining the detached leaf in prime condition on synthetic media in the view of incorporating these cultural methods into the final biosatellite unit.

Axenic Cultural Techniques.

We have also started work on developing an axenic cultural method in the event these experiments may have to be performed under suboptimal light conditions. Glassware and chemicals have been purchased for this phase of work. Presently a modified Murashige-Skoog media is being tried to culture rooted primary leaves. Leaves have been kept alive and cultures sterile for as long as a week before the death of leaves by the invasion of fungi into the petri dishes. Because of these results, the poor aseptic conditions of the laboratory due to a drafty building is being improved and we

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hope to alleviate this condition by the use of sterile hoods and culture chambers.

Possible New Recording Technique.

In the aspect of recording, the time lapse photographic method has been developed to a high reliability stage. We at U.C.L.A. have pursued other methods of recording and are now developing a system using strain guages as the recording sensor. Dr. R. D. Chipman of the U.C.L.A. Engineering Department has advised us on this project. So far, the strain gauge system has shown great promise as a recording method and we are working to overcome a minor problem of finding the proper adhesive between the plant and the metal sensor.

NEUROPORA CRASSA. 21863

Neurospora has been cultured on a low nutritional media in a 11 x 160 mm pyrex race tube. This has resulted in the desired reduction of the growth rate to 1/2" to 3/4" per day when placed in a constant temperature of $20^{\circ}C \pm 1^{\circ}C$.

A two (2) pound biopack has been developed in our laboratory for orbital flight. This biopack is 2 1/2 inches in diameter and 7 inches long (Fig. 4).

Simulated Flight Stresses.

We have made simulated orbital runs with our biopack. The innoculated race tubes were placed into the biopack, and were then subjected to flight stresses. Vibration test was used to simulate booster stresses and stresses up to 7 1/2 G's at 2 KC were given. These vibration tests were conducted at Douglas Aircraft Company under the supervision of Mr. T. Serrena (Fig. 5). All components of the biopack, the temperature control unit, and the fungus cultures did not show signs of damage from the vibration tests. The growth and banding appeared normal.

Variations in Growth Rate.

In other experiments, variation in the growth rate has been noted, where one of 6 tubes may suddenly grow out at a high rate and the band appears to be at least 4 inches in width instead of the usual 3/4" width. Further testing is anticipated to check this problem.

PROJECTED WORK ON NEUROSPORA

Future work will be concerned with the culturing of neurospora cultures on the Nogravatron to check growth patterns and test the variation in band formation under simulated weightlessness and stationary conditions.

PROJECTED WORK ON PINTO BEANS

Experimental work to be done in the second half of the year will include the observations of leaf movements while the plant is being rotated in the Nogravatron. The leaf movements under these conditions will be compared to the stationary controls. With the results we hope to obtain, a firmer base line can be established to compare the rhythmicity of the leaf under a weightlessness environment, to one under a normal gravity condition and to one under a simulated weightlessness condition.

Further work will be done on the problem of packaging the experiment. For the photographic technique, a container having a panel that will remain clear of moisture will have to be developed. The use of some heating element on the surface is contempleted. More extensive tests for vibration and gravity stresses are planned for the future.

ATLAS BARLEY SEEDLINGS, COLEOPTILE

-·-·-·- ROTATED   ·——· STATIONARY

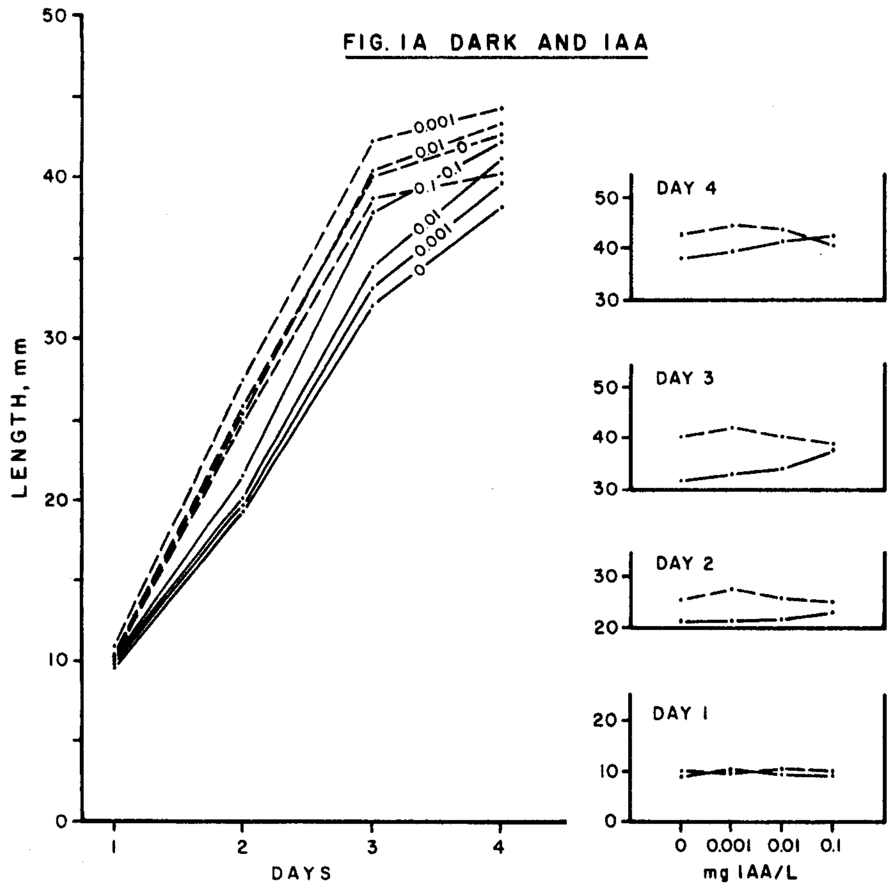


FIG. 1A EFFECT OF SIMULATED WEIGHTLESSNESS BY ROTATION AND APPLICATION OF THE PLANT HORMONE INDOLE ACETIC ACID (IAA) ON THE COLEOPTILE GROWTH OF ATLAS BARLEY SEEDLINGS GROWN IN DARK FOR 4 DAYS. LEFT GRAPH DEPICTS GROWTH VERSUS TIME AND THE RIGHT GRAPHS DEPICT GROWTH RESPONSE TO VARIOUS CONCENTRATIONS OF IAA. USUAL GROWTH OCCURRED IN STATIONARY CONTROLS WHILE THERE APPEARS TO BE DECREASED GROWTH WITH IAA IN COLEOPTILES GROWN IN A SIMULATED WEIGHTLESSNESS CONDITIONS. PLANTS WERE ROTATED 1.0 RPM ABOUT ONE AXIS AND 0.25 RPM ABOUT A SECOND AXIS PERPENDICULAR TO THE FIRST AXIS.

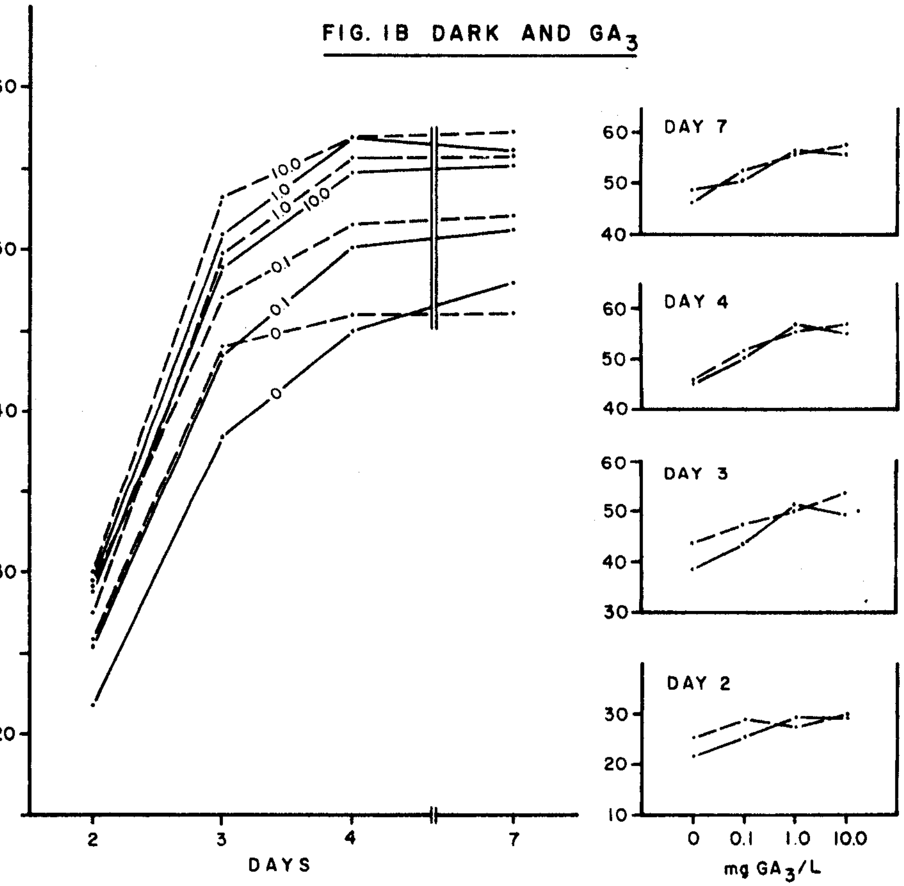


FIG. 1B SIMILAR EXPERIMENTAL TREATMENT AS IN FIG. 1A, BUT WITH THE PLANT HORMONE GIBBERELLIC ACID (GA_3) APPLIED TO SEEDLINGS. COLEOPTILE GROWTH RESPONSE TO GA_3 IS NOT MODIFIED BY A SIMULATED WEIGHTLESSNESS ENVIRONMENT. USUAL GROWTH IS STILL PRESENT IN COLEOPTILES GROWN IN A SIMULATED WEIGHTLESSNESS ENVIRONMENT.

ATLAS BARLEY SEEDLINGS

- - - - ROTATED —— STATIONARY

FIG. 2 SIMILAR EXPERIMENTAL TREATMENT AS IN FIG. 1A, BUT WITH PLANTS GROWN IN 400 FT. C. OF FLUORESCENT LIGHT. FIG. 2A - COLEOPTILE GROWTH RESPONSE TO IAA IS SIMILAR TO DARK GROWN COLEOPTILE, HOWEVER, LENGTH OF COLEOPTILE IS ABOUT 30% GREATER THAN THAT OF THE STATIONARY CONTROLS. FIG. 2B - FIRST LEAF GROWTH RESPONSE IS SIMILAR TO COLEOPTILE RESPONSE TO IAA. LENGTH OF ROTATED LEAF IS ABOUT 100% GREATER THAN STATIONARY CONTROL COLEOPTILES.

FIG. 2A LIGHT AND IAA

COLEOPTILE

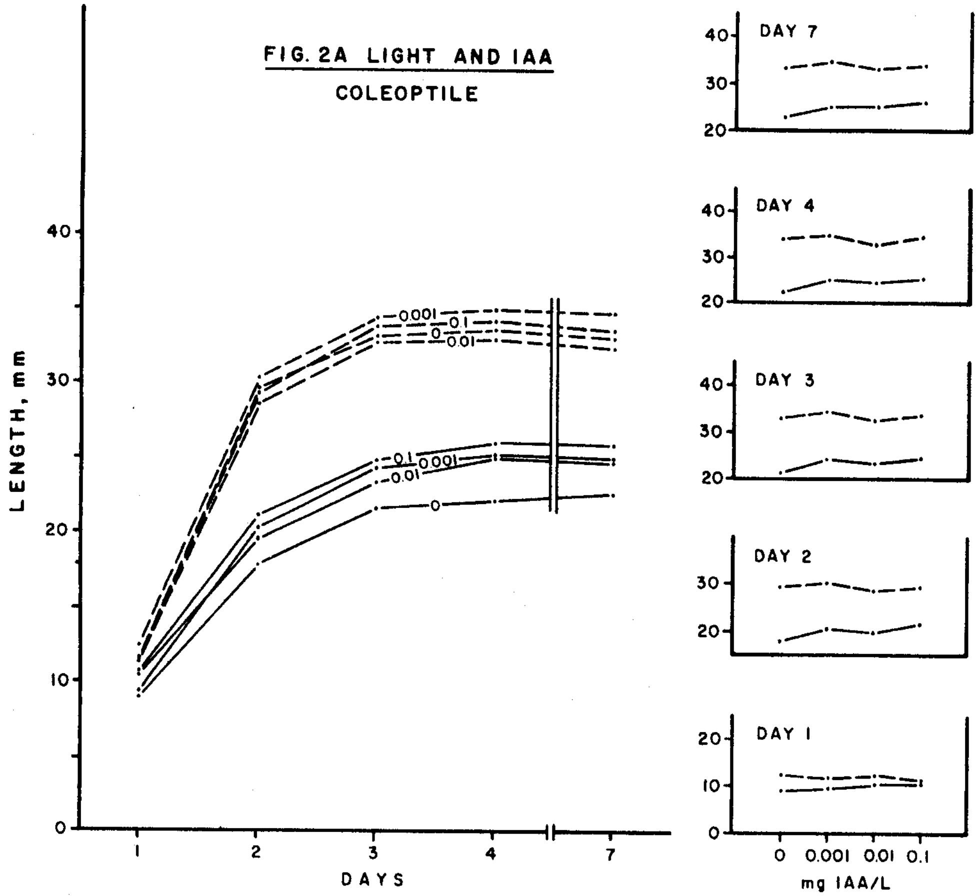
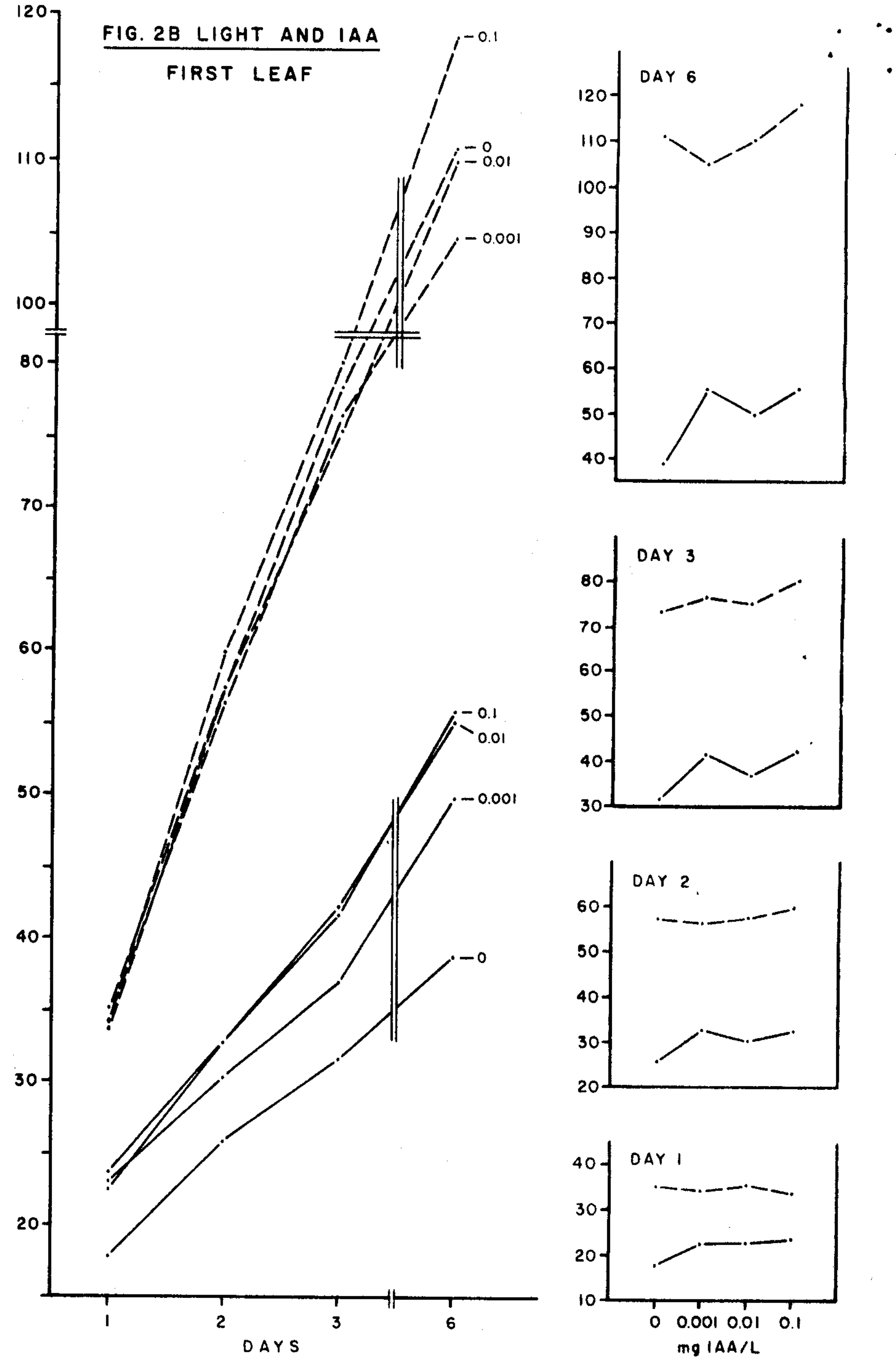


FIG. 2B LIGHT AND IAA

FIRST LEAF



ATLAS BARLEY SEEDLINGS

---- ROTATED ——— STATIONARY

FIG. 3 SIMILAR LIGHT AND SIMULATED WEIGHTLESSNESS TREATMENT AS IN FIG. 2, BUT GA_3 WAS APPLIED INSTEAD OF IAA. GROWTH RESPONSE TO GA_3 OF COLEOPTILE AND FIRST LEAF IS SIMILAR TO DARK GROWN GA_3 TREATED SEEDLINGS, HOWEVER LENGTHS OF ROTATED COLEOPTILE AND FIRST LEAF WERE ABOUT 25% AND 100% RESPECTIVELY LONGER THAN STATIONARY CONTROLS.

FIG. 3A LIGHT AND GA_3

COLEOPTILE

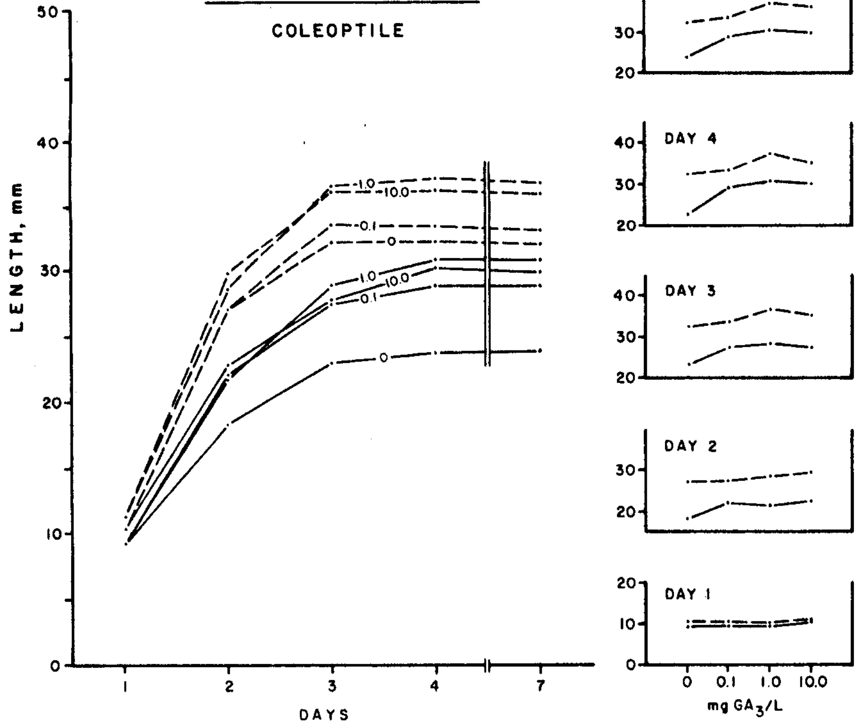
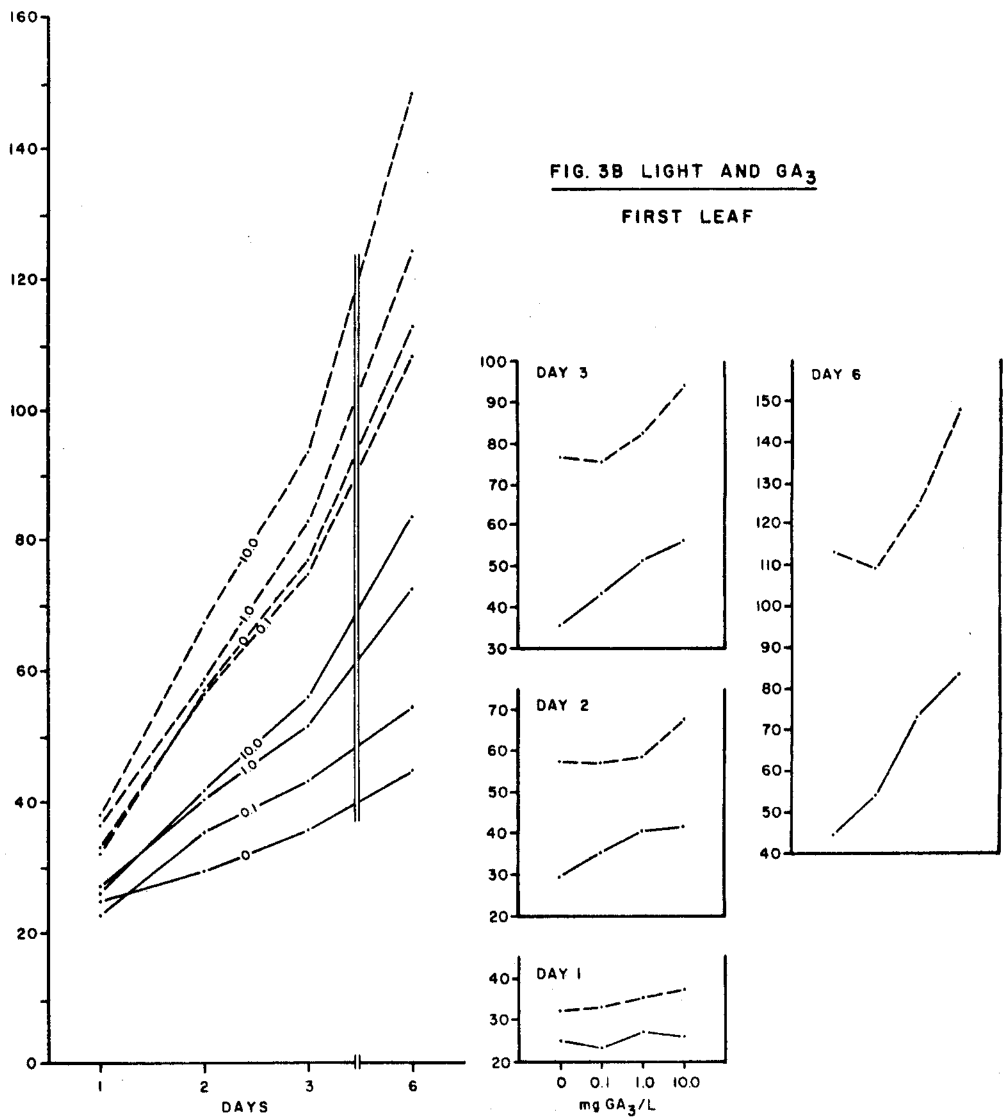
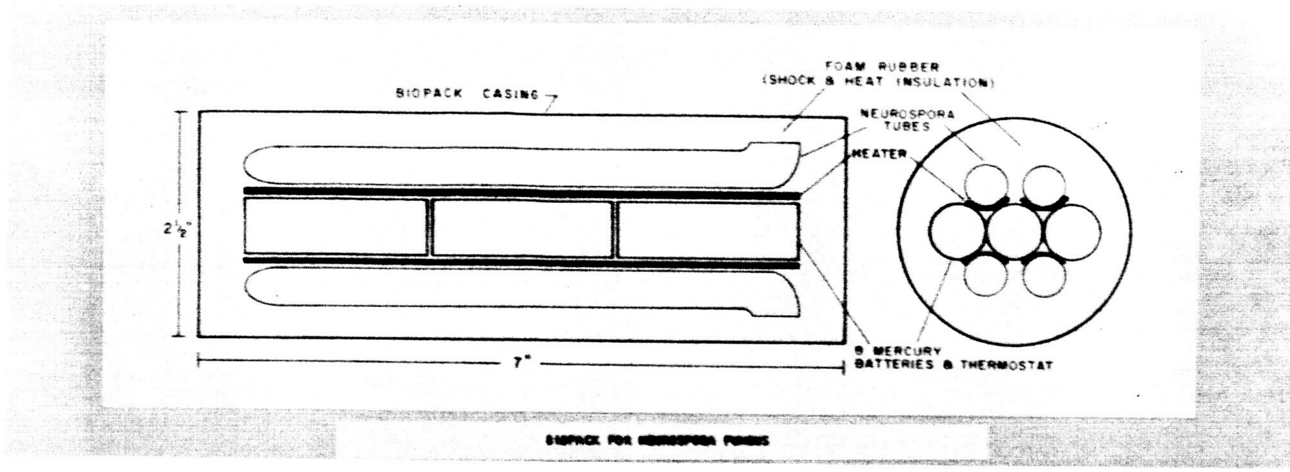


FIG. 3B LIGHT AND GA_3

FIRST LEAF





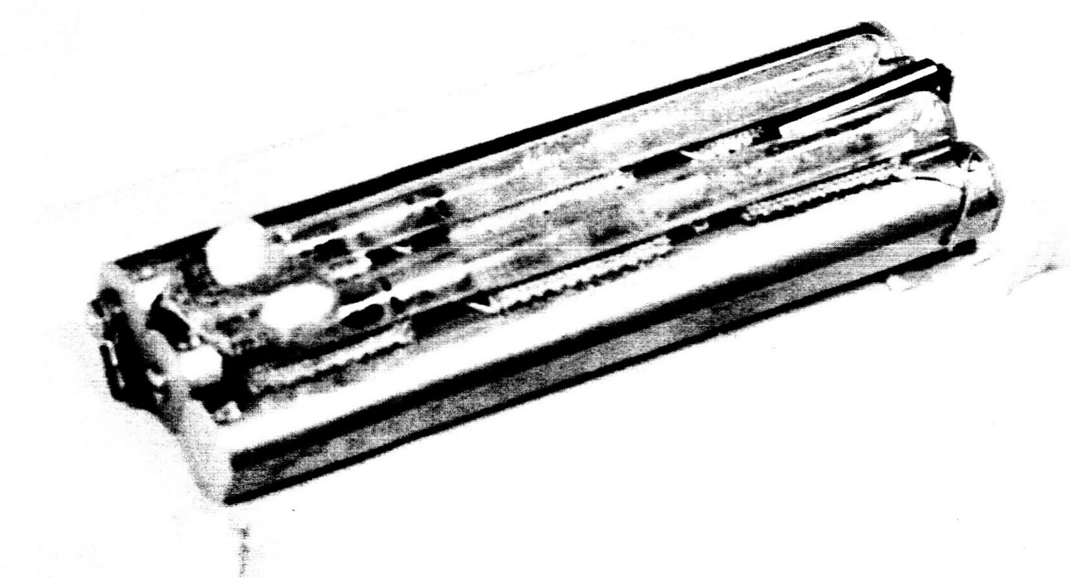


Figure 4. Biopack for 4 fungus race tubes. Thermostatically controlled heating system holds temperature 19.0°C to 22.5°C with an 8°C to 23°C environment. Total weight of unit is 2 pounds.

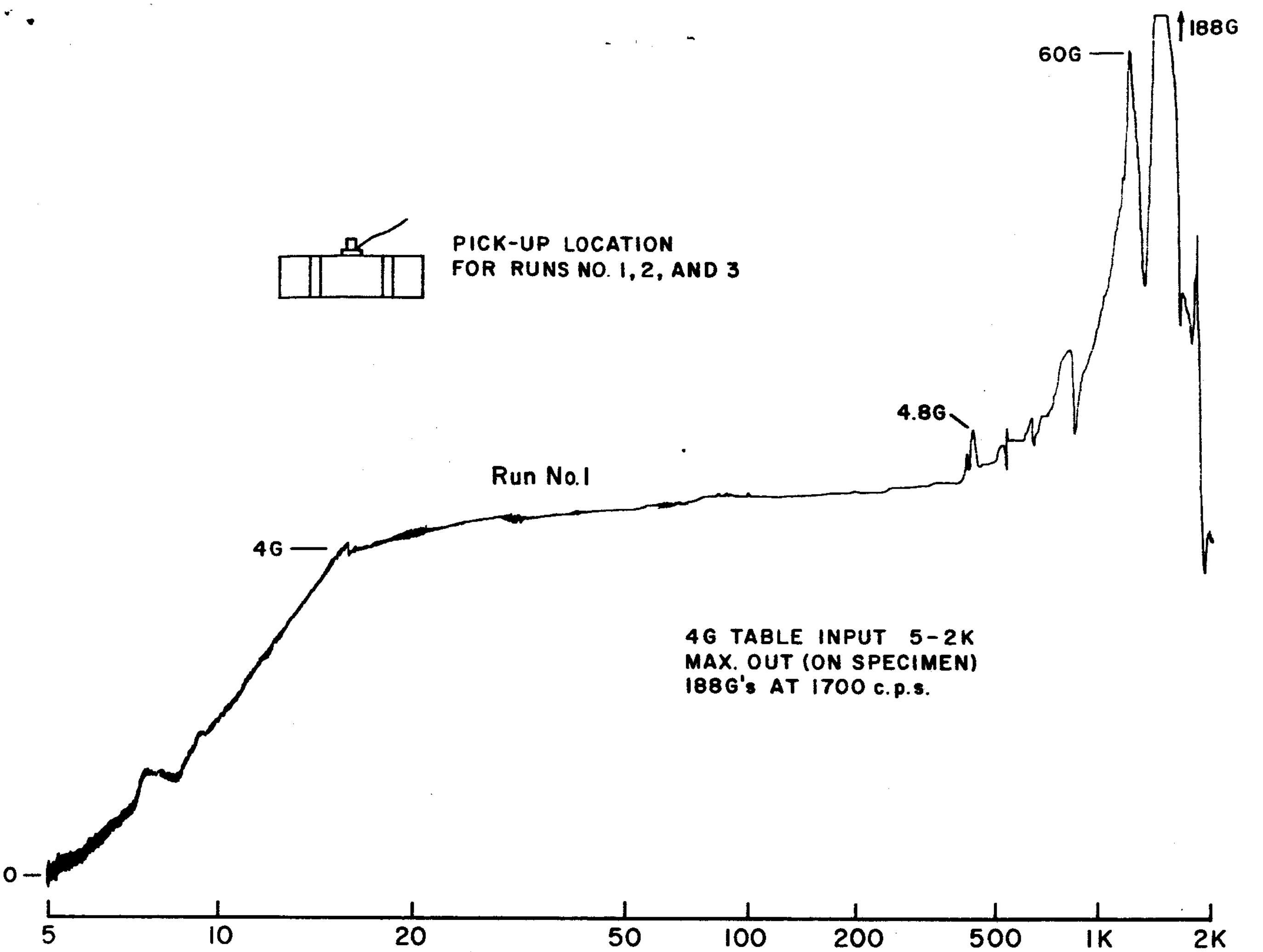


Figure 5. Data from vibration tests given to biopacks containing 4 fungus race tubes and heating system. Maximum stress measured on package was 300G at 1700 c.p.s. All components of the biopack including the fungus were not damaged by the vibration treatment.